EFFECTS OF HIV-1 INFECTION ON BONE GROWTH IN HIV-1 TRANSGENIC RATS

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Alexandra G. Cavallaro

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EFFECTS OF HIV-1 INFECTION ON BONE GROWTH IN HIV-1 TRANSGENIC RATS

Approved by:

Dr. Robert E. Guldberg, Advisor
School of Mechanical Engineering
Georgia Institute of Technology

Instr. Raja Schaar
School of Biomedical Engineering
Georgia Institute of Technology

Dr. Esfandiar Behravesh
School of Biomedical Engineering
Georgia Institute of Technology

Date Approved: 1 December 2015

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LIST OF SYMBOLS AND ABBREVIATIONS

CT	Computed Tomography
HIV	Human Immunodeficiency Virus
WT	Wild Type
AIDS	Acquired Immunodeficiency Syndrome
HAART	Highly Active Antiretroviral Therapy
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
Tg	Transgenic
pMMA	Polymethyl methacrylate
Tb.Th	Trabecular Thickness

ABSTRACT

Human immunodeficiency virus (HIV) is a subgroup of a retrovirus that causes acquired immunodeficiency syndrome (AIDS), which is a progressive failure of the immune system.[6] Millions of dollars have been donated for the research of HIV and how the virus works and effects the different systems of the body. HIV infects cells that are crucial components to the immune system and therefore eventually leads to the loss of cell-mediated immunity if left untreated.[6]

The structure of the HIV retrovirus consists of two copies of single-stranded RNA, which produces the nine proteins that HIV expresses. Since HIV enters a wide range of cells, multiple tissues are negatively altered. Previous research determined the HIV-1 Transgenic (Tg) rat is an appropriate model for experimentation due to the fact that HIV-1 viral genes they expressed as well as the immune system's response to outside invaders was very similar to humans.

To determine the effects of HIV proteins, bone histomorphometry was analyzed by taking micron samples of the harvested cortical bones. Comparing the fluorescent bands in the cortical bone allowed the bone formation rate to be determined. CT (computed tomography) scans were taken while rats were still alive to give a visual representation of the bone. From evaluating microCT scans, data was collected to prove the individual cortical bone measurements in HIV-1 Tg rats are significantly less than the control WT rats.

CHAPTER 1

INTRODUCTION

35 million people worldwide currently living with Human immunodeficiency virus (HIV) and of those, 3.2 million are children.[9] Another staggering fact, the vast majority of these people, specifically 71%, are in low and middle-income countries, as seen in the Figure 1. Human immunodeficiency virus affects 1.1 million people in the US, yet not much is known about the specific effects of HIV on the bone remodeling process.[9]

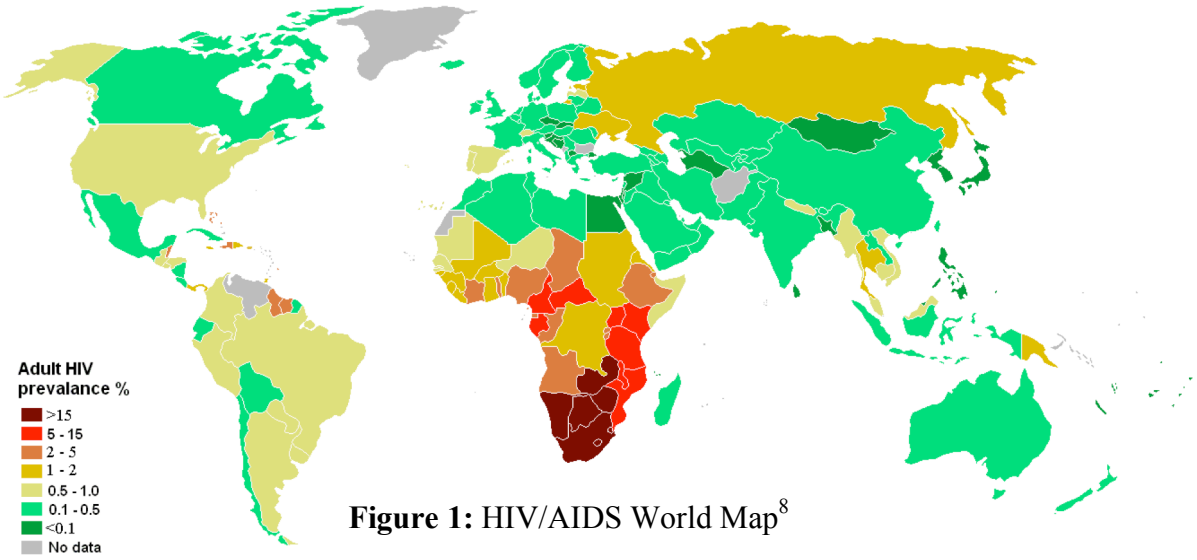

Figure 1: HIV/AIDS World Map[8]

HIV Structure And Function

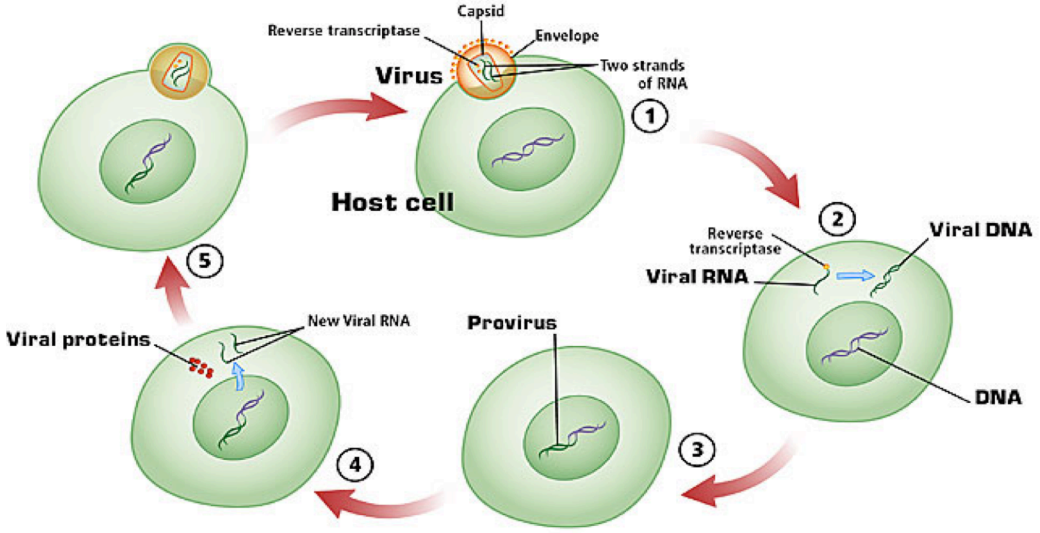

Figure 2: The replication cycle of HIV[10]

HIV is the virus that causes AIDS or acquired immunodeficiency syndrome which is the final stage of the HIV infection. Together, these cause "one of the world's most serious health and developmental challenges" (Global Statistics). This is due to the HIV virus's complex structure and life cycle, which can be seen in Figures 2 and 3. HIV can be categorized into the group of viruses called retroviruses, meaning it contains the enzyme reverse transcriptase. This enzyme "allows the single-stranded RNA of the virus to be copied and double-stranded DNA to be created" (The Structure and Life Cycle of HIV). HIV begins its invasion in the body by binding to the host cell, fusing with the host's cell membrane and finally injecting its RNA and enzymes into the cell.[10] From there, the HIV virus's

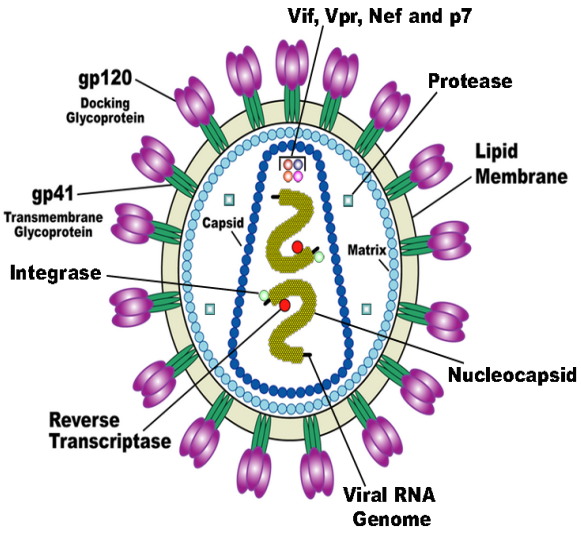

Figure 3: HIV cell diagram[7]

DNA (provirus) gets replicated within the host's cell, allowing the virus to invade and reproduce seamlessly. The HIV virus can now conquer helper T cells (or T lymphocytes, their main target) and eventually monocytes and macrophages.[10]

There are three main ways HIV is spread: sexual contact, blood-to-blood contact, and passage from mother to child. Preventing the transmission of HIV is the only way to stop the spread and consequences of HIV/AIDS.[8]

Signs And Symptoms

HIV symptoms are unique person to person, so one cannot simply rely on symptoms to know if they have HIV. The only way to confirm that one is infected with HIV is to get a blood test.[8] Some common symptoms within 2-4 weeks after exposure include flu-like symptoms: fever, sore throat, rash, swollen glands, fatigue and muscle and joint pain.[8]

Medication And Treatment

On the market today, around 30 Highly Active Antiretroviral Therapy (HAART) drugs are used to treat HIV, depending on the life cycle of the virus. There is no cure for HIV and taking one drug by itself will not stop HIV, so 'cocktails' or combinations of drugs (usually 3) are utilized.[8]

Further Research

Bioengineering research is needed in this developing field because the growth patterns of the skeleton in individuals infected with HIV, specifically in children, are unclear. Based on previous studies, children infected with HIV-1 have low bone density and display the negative effects of bone remodeling. HIV-1 transgenic rats serve as a small animal model for HIV infected individuals. Although the animals do not carry the virus, they do carry 7 of the 9 HIV genes and present with similar clinical AIDS-like symptoms by 9 months of age.[3] The study

performed seeks to determine the effects of HIV-1 proteins on the HIV-1 transgenic rats' bone growth and delicacy before all symptoms of HIV-1 appear.[1] The study also investigates the hypothesis in which HIV-1 proteins are directly correlated with negative effects on skeletal growth patterns and bone repair.

CHAPTER 2

LITERATURE REVIEW

To aid in the background of my research, multiple sources of literature were accessed and analyzed to provide a foundation and jumping off point for my study. From Wong's paper, he notes that the "HIV group had significantly lower values for all cortical bone parameters except average mineralization compared to the WT group," which directly correlates with my research of HIV transgenic rat bone growth and gives me a foundation to build on.[1]

Vikulina writes another article that highlights the bone growth differences, mainly the effects/complications of HIV-1- osteoporosis and bone fractures. Vikulina harps on the fact there are findings that confirm that reduced bone mineral density is an effect of HIV-1. The article goes on to target the specific molecular components of the bone remodeling process the "receptor activator of NF-kB ligand, the key osteoclastogenic cytokine, to its receptor osteoprotegerin."[3] Although complex, the molecular component that supports my current findings in my research of slowed or reduced bone growth and reduced bone density were found in HIV-1Tg rats. It also takes into account the highly active antiretroviral therapy (HAART) and how that may have an effect on the bone density and growth. Looking at bone growth in particular, HIV-1 transgenic rats undergo major bone reconstruction when infected with HIV type 1. This is mainly due to immune cells regulating the osteoclasts performance, so when the immune system is compromised with HIV, the skeleton suffers as well.[3]

In Reid's article, he presents in depth the HIV-1 transgenic rat type and how the phenotype of the rat can be applicable as a model for HIV-1 in humans. Specifically, the HIV Tg rat is very much like humans that have HIV-1 with respect to the viral genes they expressed as well as the immune system's response to outside invaders. Also noteworthy, the skin and

muscle tissues have the most HIV-1 genes found in their cells, which is why skin lesions, cataracts and other characteristics displayed in the rat are very common. The RNA in the cells is tested in many cells to find the highest organs- the lymph nodes, spleen, kidney and thymus. Knowing this information makes it easier to test animal and human subjects to monitor levels of expression.[2]

Other papers have hypothesized that as the animal changes age, the expression of the HIV proteins changes from peripheral immune organs to the central nervous system. At 2-3 months old, the rats were measured of having a much larger concentration of RNA viral protein in their spleens than rats that were 10-11 months old. However, the concentration of mRNA viral protein in the central nervous system- the cerebellum, striatum and spinal cord- in 10-11 month old rats was significantly higher than in 2-3 month of rats. Both of these tests prove that there are different viral proteins expressed at different times for different ages.[5] This concept could explain the growth patterns that I am seeing in my research. It also takes into account the highly active antiretroviral therapy (HAART) and how that may have an effect on the bone density and growth. The technical and molecular aspect of the physical actions and traits discussed in this paper would add to my research by adding an answer to growth patterns or phenotype expression.[4]

CHAPTER 3

RESULTS

The results compiled from the two years of research yields data to illustrate findings for the proposed hypothesis of slowed bone growth of HIV-1 transgenic rats. The main set of results is from examining and compiling data from vivaCT evaluations from alive 5, 7, and 9 months old rats.

Cortical Bone Thickness Comparison

From the data collected from the vivaCT evaluations, it is easy to compile the data into a simple Table 1 below to see the large difference of cortical bone thickness of the femur between the WT and HIV rats. All measurements from the WT group were averaged, then the HIV group was averaged, to create the mean cortical bone thickness displayed in the table. The average cortical bone thickness was 0.603 mm thick for WT rats and 0.570 mm thick for HIV. Since the calcein labeling didn't add any data to my research, the vivaCT evaluations were the foundation of my results.

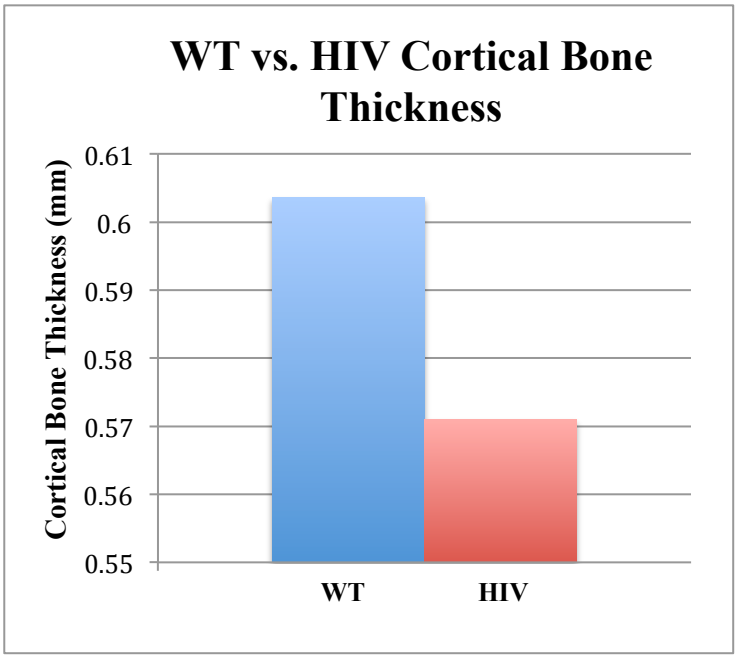

Table 1: WT vs. HIV Cortical Bone Thickness

The samples embedded in the Polymethyl methacrylate (pMMA), a synthetic resin produced from the polymerization of methyl methacrylate results were not finalized with my research, but some slices were used for calcein

labeling. The rest of the slides will be used to determine the bone morphology rate and future growth patterns.

CHAPTER 4

DISCUSSION

The results stated previously yields data to support the proposed hypothesis of slowed bone growth of HIV-1 transgenic rats. The smaller average cortical bone thickness of the HIV-1 transgenic rats indicated frailer femurs compared to the WT rat femurs. Based on background literature and contributing results, one can reasonably concur HIV-1 proteins may be expressed before symptoms are detected and influence multiple body functions at a young age. Future work will have to support these conclusions by comparing bone densities, weight, and survey a variety of ages of both HIV-1 transgenic rat and the control WT rat.[1]

Significance

This study contributes to the Guldberg lab's HIV study as a whole, which displays the changes in bone growth before all symptoms of HIV-1 appear, even though HIV-1 proteins are expressed from birth or before birth. The microCT scans that evaluated provides data and proof into the effects of HIV on bone growth patterns. The result of previous microCT scans show that the individual cortical bone measurements in HIV-1 Tg rats is significantly less than the control WT rats. The long reaching effects of the studies performed can be a foundation for clinical trials for new HIV drugs or treatments. Knowing these growth patterns can help doctors treat HIV patients according to the age and progression of HIV.

This allows all proteins to be expressed and all changes in bone growth, remodeling and regeneration to be completely tracked as well as osteoporosis data. This study would link current HIV transgenic rat studies together to see correlations between bone growth and tissue repair or organ growth. In addition, this research examines different bone measuring parameters than previously researched, which allows extension of current data and conclusions. Based on

these conclusions, this research fills the current gap in research of what effects the HIV infection has on the growth and development of the skeletal bones.

CHAPTER 5

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Georgia Institute of Technology approved all procedures. In this study, female HIV-1 Tg rats and control wild type (WT) rats were used and were purchased from Harlan Laboratories. To analyze bone growth, the Tg rats were scanned using a vivaCT system from Scanco Medical, at 5, 7, and 9 months old. Histological sample processing, examination of calcein labeling, and vivaCT reconstruction evaluations were used to collect data that proved the hypothesis.

VivaCT Evaluation Analysis

VivaCT evaluations were performed on the vertebrae, distal femur, and middiaphysis. To determine cortical bone parameters, a 1 mm region of the femoral middiaphysis was scanned and evaluated. The bone parameters used from the femoral middiaphysis for further quantitative data were the average mineralization, cortical bone thickness, marrow area and bone area. The average mineralization component is the measure of attenuation, or how easily x rays are passed through the material. This allows researchers to compare the bone densities between the HIV-1

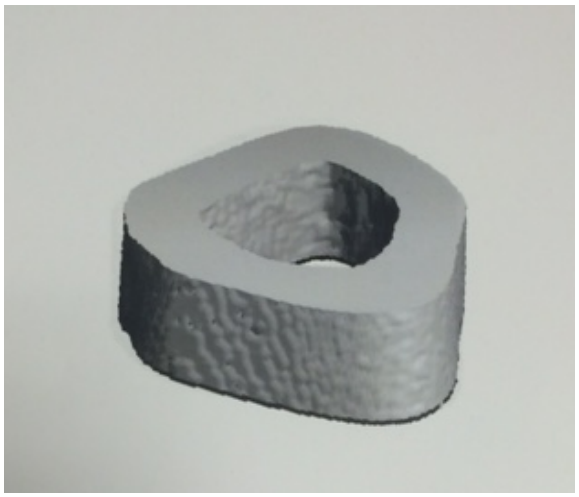

Figure 4: The vivaCT reconstruction evaluation of sample 33941 (WT)- 1mm section of middiaphysis

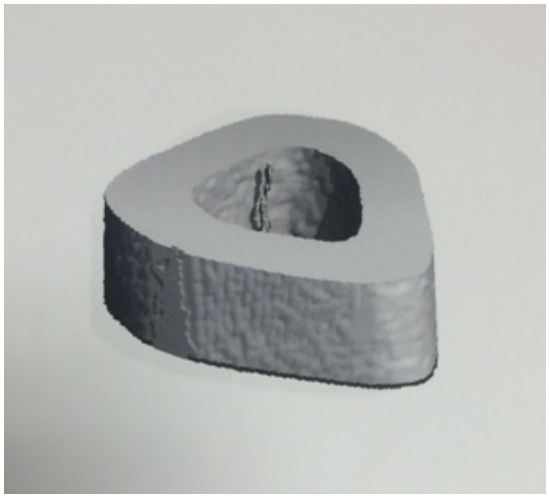

Figure 5: The vivaCT reconstruction evaluation of sample 33966 (HIV)- 1mm section of middiaphysis

Tg rats and the WT rats. The vivaCT reconstruction scans of 1mm region of the femoral middiaphysis in both the Wild Type (WT) rats and HIV rats are shown in Figures 4 and 5 above, respectively. In this analysis, a number of parameters are measured and output on the final printout of the vivaCT evaluation.

Next, the cortical bone thickness, marrow area and bone area were evaluated and measured in both WT and HIV-1 transgenic rats. The Tb.Th (trabecular thickness) component on the evaluation was used to determine the trabecular thickness (mm), and the average mineralization (in the central marrow portion) was also measured. The data is displayed in the Table 2 to the right.

Measurement Number	Thickness [mm]	Average Mineralization [mg HA/cm^3]
33556	0.5901	1331.2308
33559	0.6304	1311.5402
33565	0.6134	1309.1829
33562	0.6449	1320.9695
33626	0.5887	1307.1028
33630	0.5931	1322.2175
33669	0.6187	1323.1882
33666	0.559	1313.3429
33663	0.5945	1321.9402
33549	0.5981	1362.5696
33552	0.5686	1331.3695
33568	0.5624	1329.1509
33571	0.5817	1322.6335
33636	0.5711	1318.7509
33640	0.5731	1316.3936
33648	0.5518	1329.5669
33652	0.5838	1333.5883
33659	0.5667	1320.8308
33656	0.553	1304.7455

Table 2: Data of Trabecular Thickness and Average Mineralization of Cortical bones of HIV and WT rats

After the rats were sacrificed and the femurs and humeri were harvested, the samples were processed for histological evaluation by using a microtome to cut rat tibias embedded in blocks of pMMA (Polymethyl methacrylate). From trial and error this method written protocol did not work, so cutting pMMA block with a 5in diamond saw arose as an alternate procedure. Slices 80-200 microns thick of the sample were cut and mounted to a microscope slide and viewed under a microscope for observation. Since these slices of 80-200 microns were determined to be too thick to be analyzed, a grinding procedure was adopted to improve the

quality of the samples. The grinding procedure began by attaching one side of the sample to a 'final slide' with Technovit 7210 glue, then a 'base slide' was attached to the other side of the sample, forming a 'sandwich.' The sandwich was then cut with a 5in diamond saw to cut off a slice of a sample glued to the 'final slide' for further grinding. After the slide is measured, the slide with sample is placed on the grinder and grinded down with 1000 grit paper until the sample is an optimal 40-60 microns thick. The sample is then polished for clarity and used for further analysis and testing.

Bone Histomorphometry Analysis

Bone histomorphometry was analyzed and more specifically bone formation rate was determined by comparing the calcein-labeling fluorescent bands in the cortical bone. To create these bands, an initial round of calcein was injected into living HIV-1 transgenic and WT control rats and one week later a second round of calcein was injected again. Shortly after, the rats were sacrificed and the femurs from both HIV and WT control rats were harvested. The femurs were then embedded in pMMA for histologic analysis. From there, the 80-100 micron slices of the femurs were taken and placed on microscope slides. A green excitation light in the microscope was used to see these fluorescent bands in the cortical bone. These fluorescent bands are measured to determine bone formation rate by comparing the spacing in between the bands. Most samples did not have a distinct dual labeling appearance; so only seventeen samples could be analyzed for this study. In Figures 6 and 7, dual labeling can be seen with two layers of distinct bands. In both images, the labeling is spaced the same, so it was determined that the calcein labeling was not a good comparison because no differences were seen.

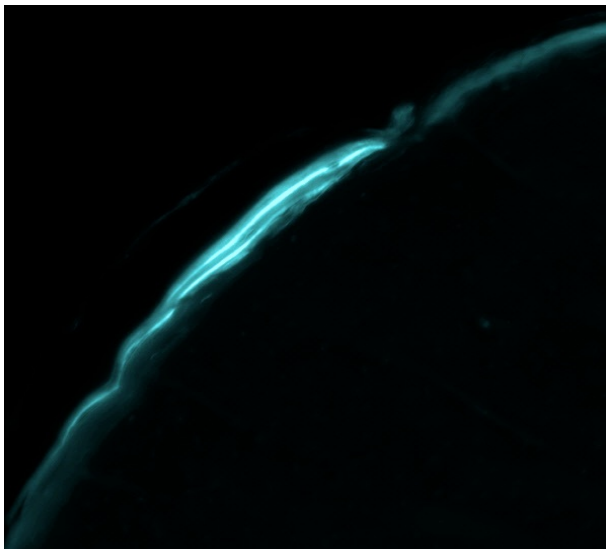

Figure 6: Calcein dual labeling in sample 2101 (WT) on 1/10/14

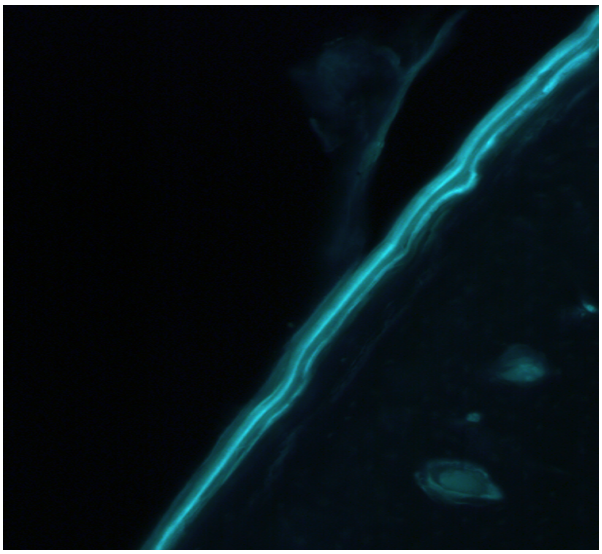

Figure 7: Calcein dual labeling in sample 2110 (HIV) on 1/10/14

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